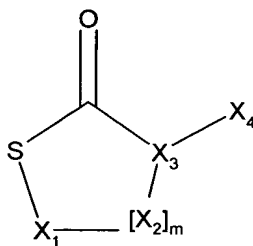


What is claimed is:

1. A method of generating at least one polydentate metal chelating affinity ligand, which method comprises the steps of

- 5 (a) providing at least one scaffold defined by a ring of the general formula (I)



wherein

X<sub>1</sub>, X<sub>2</sub> and X<sub>3</sub> irrespective of each other are sp<sup>2</sup>- or sp<sup>3</sup>-hybridised carbon atoms or heteroatoms,

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X<sub>4</sub> is a nucleophile and

m is an integer of 0-2;

- (b) providing at least one polydentate metal chelating affinity ligand arm on each scaffold by derivatisation of the nucleophilic X<sub>4</sub> groups of said scaffolds while retaining the cyclic structure of the scaffold; and
- 15 (c) opening the ring at a bond between the carbonyl and the sulphur of the derivatised scaffold by adding a reagent that adds one or more metal chelating affinity ligand arms to the scaffold.

- 20 2. The method of claim 1, wherein in formula (I), X<sub>1</sub>, X<sub>2</sub> and X<sub>3</sub> are carbon atoms.

3. The method of claim 1, wherein in formula (I),  $X_4$  is  $-NH_2$ .
4. The method of claim 1, wherein in formula (I), m is 1 and the scaffold is  
5 homocysteine thiolactone.
5. The method of claim 1, wherein in step (b), the derivatisation is provided by  
adding at least one derivatisation agent comprised of one part, which is  
electrophilic and hence capable of reacting with  $X_4$  in Formula (I), and a  
10 second part, which is a metal chelating affinity ligand.
6. The method of claim 5, wherein the derivatisation is provided by adding two  
derivatisation agents, which comprise two different or identical metal  
chelating functionalities.
- 15 7. The method of claim 5, wherein said at least one derivatisation agent is a  
halogenated, protected ester.
8. The method of claim 7, wherein said at least one derivatisation agent is  
20 bromo-acetic acid ethyl ester.
9. The method of claim 1, wherein in step (c), ring-opening is hydrolysis  
provided by adding a base.

10. The method of claim 1, wherein the metal chelating affinity ligand arms are protected in step (b).
11. The method of claim 10, further comprising the steps of deprotecting the  
5 chelating affinity ligand arms after step (c) and coupling the resultant compound via its thiol group to a base matrix in order to produce a separation medium.
12. The method of claim 11, wherein the thiol group is coupled to allyl groups of  
10 the base matrix.
13. The method of claim 11, further comprising a step of allylating the base matrix to provide reactive groups to permit said coupling.
14. The method of claim 11, wherein the thiol group of the ligand is coupled to the  
15 base matrix via an allyl group of allyl glycidyl ether (AGE).
15. The method of claim 11, further comprising a step of activating the reactive groups of the base matrix.
- 20 16. The method of claim 15, wherein the activating is performed by bromination.
17. A polydentate metal chelating affinity ligand, or a separation medium comprising a plurality of polydentate metal chelating affinity ligands coupled  
25 to a base matrix generated by the method of claim 1.

18. The ligand or separation medium of claim 17, wherein the ligand is a tridentate ligand.
- 5 19. A kit which comprises the scaffold of claim 1, which kit includes said scaffold in a solid state together with written instructions for use thereof in the manufacture of a separation medium including a plurality of polydentate metal chelating affinity ligands coupled to a base matrix.
- 10 20. The kit of claim 19, wherein the scaffold is homocysteine thiolactone.
21. A separation medium comprising polydentate metal chelating affinity ligands coupled to a base matrix and defined by the general formula base matrix–O–CH<sub>2</sub>–CHOH–CH<sub>2</sub>–O–CH<sub>2</sub>–CHOH–CH<sub>2</sub>S – (CH<sub>2</sub>)<sub>n</sub> – CH(COOH) –  
15 N(CH<sub>2</sub>COO<sup>–</sup>)<sub>2</sub>Ni<sup>2+</sup> wherein n is an integer of 2-4.
22. The medium of claim 21, wherein n = 2.
23. A chromatography column for immobilised metal ion adsorption  
20 chromatography (IMAC), packed with the separation medium of claim 17.
24. A process of separating a target substance from a liquid, which process comprises providing the separation medium of claim 17 to charge said medium with suitable metal ions to form chelates and to contact said medium  
25 with the liquid to adsorb the target substance thereon.

25. The process of claim 24, further comprising a step of eluting the target substance from the separation medium by adding a liquid that desorbs the target compound from the separation medium.

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26. The process of claim 25, wherein the eluting is obtained by use of a liquid that comprises a decreasing pH gradient or by applying a gradient of increasing imidazole concentration.

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